

Please substitute the following paragraph on page 4, line 25 through page 5, line 3:

Membrane transporter proteins and ion channel proteins serve critical roles in maintaining organic solute and ionic metabolic, thermodynamic, and electrical events in all cells. In both eukaryotes and prokaryotes these proteins affect electrochemical gradients of a wide variety of metabolic molecules and electrolytes, including amino acids and related metabolites as well as H^+ , OH^- , Na^+ , K^+ , Cl^- , and carbonate ions (Gerencser and Stevens, 1994, *J. Exper. Biol.* 196:59-75; Stevens, B.R. 2001. "Theory and methods in nutrient membrane transport." In: *Surgical Research*. pp. 845-856. W.W. Souba and D.W. Wilmore, eds., Academic Press, San Diego). Molecular cloning studies have identified several subfamilies of organic solute transporters and ion channels (Griffith, J.K. and C.E. Sansom, 1998, In: *The Transporter Facts Book*, Academic Press, San Diego, pp. 500).

Please substitute the following paragraph on page 5, lines 4-20:

Organic solute transporters and ion channels are commonly defined by their substrate selectivity within polypeptide superfamilies. For cloned or native secondary active transporters, it is generally assumed that cell membranes utilize ion and organic molecule electrochemical gradients to aid in exchanging these solutes between the cell interior and extracellular environment (Gerencsér, G.A. and B.R. Stevens, 1994, *J. Exper. Biol.* 196:59-75; Stevens, B.R. 1999, *Digestion and Absorption of Protein*. In: *Biochemical and Physiological Aspects of Human Nutrition*. pp. 107-123, M.H. Stipanuk, ed., W.B. Saunders Co., Philadelphia). In the 'prototypical' transporter, organic solutes that can be moved across cell membranes by uniport, hetero- or homo-exchange, and/or uptake can be activated by ions, and/or thermodynamically cotransported with ions (Quick, M. and B.R. Stevens, 2001, *J. Biol. Chem.* 276(36):33413-33418; Griffith, J.K. and C.E. Sansom, 1998, In: *The Transporter Facts Book*, Academic Press, San Diego, pp. 500). Ion channels, on the other hand, are typically distinct from organic solute transporters, are selective in their conducting ion species, and may be gated by organic ligands (Hille, B, 2001, *Ionic channels of excitable membranes*, 3rd Edition, Sinauer Associates, Inc., Sunderland, Mass., pp. 814).

Please substitute the following paragraph on page 6, lines 1-11:

The midgut region of *M. sexta* larvae displays compartments with the property of high concentrations of K⁺ as well as Na⁺ in an alkaline fluid (~pH 10), with trans-epithelial potentials ~250 mV (Harvey *et al.*, 1999, *Am. Zool.* 38:426-441; Harvey and Wieczorek, 1997, *J. Exper. Biol.* 200:203-216). Epithelial cells of this region transport a variety of nutrients, including nutrient amino acids and electrolytes, as demonstrated by *in vitro* isolated membrane vesicle uptake studies. In place of a Na⁺/K⁺-ATPase typically found in cells, this tissue instead possesses a proton translocating V-ATPase (Graf *et al.*, 1992, *FEBS Lett.* 300:119-122; Merzendorfer *et al.*, 1997, *J. Exper. Biol.* 200:225-235) which energizes the cell membranes for secretion and absorption of K⁺ and Na⁺ ions, and establishment of a large pH gradient. A K⁺-activated leucine-preferring transporter (KAAT1) has been identified from the hornworm midgut (Castagna *et al.*, 1998, *Proc Natl. Acad Sci. USA* 95:5395-5400), and a GABA (gamma aminobutyric acid) transporter has been cloned from an *M. sexta* embryo cDNA library (Mbungu *et al.*, 1995, *Arch. Biochem. Biophys.* 318:489-497).

Please substitute the following paragraph on page 22, lines 23-29:

The subject invention further contemplates the use of peptide nucleic acids (PNAs) in the practice of the methods of the invention. PNA is a DNA mimic in which the nucleobases are attached to a pseudopeptide backbone. PNA may be utilized in a number of methods that traditionally have used RNA or DNA. Often PNA sequences perform better in techniques than the corresponding RNA or DNA sequences and have utilities that are not inherent to RNA or DNA. A review of PNA including methods of making, characteristics of, and methods of using, is provided by Corey (Corey, D.R., 1997, *Trends Biotechnol.* 15:224-229) and is incorporated herein by reference.

Please substitute the following paragraph on page 28, lines 1-9:

In other embodiments, it is contemplated that certain advantages will be gained by positioning the coding DNA segment under the control of a recombinant, or heterologous, promoter. The use of promoter and cell type combinations for protein expression is generally known to those of skill in the art of molecular biology, for example, see Maniatis *et al.*, 1989 (Sambrook, J., E.F.

Fritsch and T. Maniatis, 1989, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, New York). The promoters employed may be constitutive, or inducible, and can be used under the appropriate conditions to direct high level expression of the introduced DNA segment, such as is advantageous in the large-scale production of recombinant proteins or peptides. Appropriate promoter systems contemplated for use in high-level expression include, but are not limited to, the *Pichia* expression vector system (Pharmacia LKB Biotechnology).

Please substitute the following paragraph on page 28, line 24 through page 29, line 12:

An initial set of inosine ("I")-containing degenerate primers was designed (Feldman *et al.*, 2000, *J. Biol. Chem.* 275:24518-24526) to target conserved peptide motifs from invertebrate and vertebrate members of a subfamily of Na/Cl dependent transporters serving various neurotransmitters and amino acids (Griffith, J.K. and C.E. Sansom, 1998 In: The Transporter Facts Book, Academic Press, San Diego, pp. 500). All primers are shown in the 5' to 3' direction. The sense primer "S34" (GGIAA(C/T)GTITGG(A/C)G(A/G/C/T)TT(C/T)CC) (SEQ ID NO:3) was based on a GNVWRFP (SEQ ID NO:4) peptide motif, while the antisense primer "S21" (IGC(A/G/T)ATIGCITC(A/G/C/T)GG(A/G)TA) (SEQ ID NO:5) was based on a YP(D/E)AIA (SEQ ID NO:6) peptide motif. Another sense primer "S22" (GGIAA(C/T)GTITGG(G/T)G(A/G/C/T)TT(C/T)CC) (SEQ ID NO:7), a tolerated alternative to S34, was also used in conjunction with S21 antisense primer for initial screening. Another primer set was designed to specifically exclude KAAT1 and other potentially related sequences, including, while amplifying a unique 328 bp segment. In this case, sense primer "S25" (AACACTTGCTGCATCAGTCAAC) (SEQ ID NO:8) and antisense primer "S26" (CTCAAGGAGTTCGCCCATTG) (SEQ ID NO:9). The S25/S26 set was used for subsequent library phage lysate PCR™ screening steps, and was used with the cloned 943 bp fragment to create a 328 bp digoxigenin (DIG)-labeled (Boehringer-Mannheim) dsDNA plaque hybridization probe for the isolation of a single clone. The sequence of the full length clone (SEQ ID NO:1) was determined, including the open reading frame plus the 3' and 5' untranslated regions.

Please substitute the following paragraph on page 31, lines 4-18:

The CAATCH1 nucleotide coding sequence within the full length clone of 2858 nt (SEQ ID NO:1) contained an open reading frame encoding a predicted unique polypeptide sequence of 633 amino acids (SEQ ID NO:2). The predicted amino acid sequence of CAATCH1 is compared to related members of a subfamily of membrane proteins that includes Na/Cl activated transporters of neurotransmitters (Quick, M. and B.R. Stevens, 2001, *J. Biol. Chem.* 276(36):33413-33418; Feldman *et al.*, 2000, *J. Biol. Chem.* 275(32):24518-24526; Griffith, J.K. and C.E. Sansom, 1998, In: The Transporter Facts Book, Academic Press, San Diego, pp. 500) and the *Manduca* KAAT1 leucine transporter (Castagna *et al.*, 1998, *Proc. Natl. Acad. Sci. USA* 95:5395-5400). Based on hydropathy analysis and established membrane protein structure paradigms (Kyte and Doolittle, 1982, *J. Mol. Biol.* 157:105-132), the predicted CAATCH1 protein topology includes 12 putative transmembrane domains, with N- and C- terminal segments residing within the cytosol. Several consensus phosphorylation sites are found within these cytoplasmic segments, and N-linked glycosylation sites exist on the putative extracellular loop between the 3rd and 4th membrane spanning segments. The cytosolic N- and C- terminal regions are relatively rich in proline, acidic, and basic amino residues.

Please substitute the following paragraph on page 31, line 19 through page 32, line 16:

Membrane transporters and ion channels in general can be subcategorized based on thermodynamic properties, substrate selectivities, and reaction mechanism (Gerencser, G.A. and B.R. Stevens, 1994, *J. Exper. Biol.* 196:59-75; Hille, B., 2001, In: Ion Channels of Excitable Membranes, 3rd Edition, Sinauer Associates, Inc., Sunderland, Mass, pp 814). CAATCH1 cloned from *Manduca sexta* collectively displayed a unique set of properties (Quick, M. and B.R. Stevens, 2001, *supra*; Feldman *et al.*, 2000, *supra*) that have not been described previously for a given related transporter, including at least the following attributes. These same attributes are also assigned to both *Aedes aegypti* and *Leptinotarsa decemlineata* by inference of their sequences being identical to SEQ NO:1. The attributes include: (a) the ability to switch particular amino acid substrate selectivities depending on the activator cation Na⁺ or K⁺, (b) a unique selectivity profile of amino acid-evoked electrical currents, (c) different amino acid substrates directly binding the protein and differentially affecting the conformational states of CAATCH1, apparent lack of chloride ion as a co-activator, (d)

Nernstian cation channel behavior independent of amino acid transporter activity, (e) amino acid-modulated ion channel behavior (especially L-methionine binding to CAATCH1 in the presence of Na^+ which perturbs the charge-voltage relation with a high affinity binding constant, affecting transient currents due to CAATCH1-associated charge transfer across the membrane dielectric field), (f) inhibition of current fluxes as the result of binding of methionine to the protein, (g) thermodynamically uncoupled amino acid transport and ion channel behavior, and (h) all these functions behave optimally at an alkaline pH. CAATCH1 defines a new transport system (Quick, M. and B.R. Stevens, 2001, *supra*; Feldman *et al.*, 2000, *supra*; Castagna *et al.*, 1998, *supra*; Christensen, H.N., 1990, *Physiol. Rev.* 70:43-77; Griffith and Sansom, 1998, *supra*; Kilberg *et al.*, 1993, *Annu. Rev. Nutr.* 13:137-165; Mailliard *et al.*, 1995, *Gastroenterology* 108:888-910; Malandro and Kilberg, 1996, *Annu. Rev. Biochem.* 65:305-336; Stevens, B.R., 1992, "Amino Acid Transport in Intestine" In: *Mammalian Amino Acid Transport: Mechanisms and Control*, pp 149-164, M.S. Killberg and D. Haussinger, eds., Plenum, New York). The transport of amino acids by CAATCH1 serves the simultaneous and independent roles of nutrient transporter and amino acid-gated ion channel in *M. sexta*, *A. aegypti*, or *L. decemlineata*.

Please substitute the following paragraph on page 52, lines 5-21:

Genes encoding pesticidal compounds, as disclosed herein, can be inserted into plant cells using a variety of techniques that are well known in the art. For example, a large number of cloning vectors comprising a replication system in *E. coli* and a marker that permits selection of the transformed cells are available for transforming higher plants, e.g., pBR322, pUC series, M13mp series, pACYC184, etc. Accordingly, the sequence encoding the pesticidal peptide can be inserted into the vector at a suitable restriction site. The resulting plasmid can be used for transformation into *E. coli*. The *E. coli* cells can be cultivated in a suitable nutrient medium, then harvested and lysed. The plasmid can be recovered. Sequence analysis, restriction analysis, electrophoresis, and other biochemical and/or molecular biological methods can be generally carried out as methods of analysis. After each manipulation, the DNA sequence used can be cleaved and joined to the next DNA sequence. Each plasmid sequence can be cloned in the same or other plasmids. Depending on the method of inserting desired genes into the plant, other DNA sequences may be necessary. If, for

example, the Ti plasmid (the tumor-inducing plasmid of the plant-pathogenic bacterium *Agrobacterium tumefaciens*) or Ri plasmid (the root-inducing plasmid of *Agrobacterium rhizogenes*) can be used for the transformation of the plant cell, then at least the right border, but often the right and the left border of the Ti or Ri plasmid T-DNA ("Transferred DNA"), must be joined as the flanking region of the genes to be inserted.

Please substitute the following paragraph on page 53, lines 1-13:

A region referred to as the T-DNA ("Transferred DNA") is transferred from an infecting *Agrobacterium* cell into the nucleus of the plant cell, where it is integrated into the plant genome. The use of T-DNA for the transformation of plant cells has been intensively researched and sufficiently described in Eur. Pat. Appl. No. EP 120 516; Hoekema (*In: The Binary Plant Vector System*, Offset-durkkerij, Kanters B.V., Albllasserdam, Chapter 5, 1985); An *et al.* (*EMBO J.* 4:277-287, 1985); Herrera-Estrella *et al.* (*Nature* 303:209, 1983); Bevan *et al.* (*Nature* 304:184, 1983); and Klee *et al.* (*Bio/Technology* 3:637-642, 1985). Transfer of the T-DNA depends on a set of genes called *vir* if they are on the Ti plasmid, or *chv* if they are on the chromosome. These genes are induced in response to various compounds in exudates from wounded plants. The T-DNA itself is flanked by repeated sequences of around 25 base pairs, called border repeats (or left and right borders). The T-DNA contains a group of genes referred to as the *onc* genes, which are responsible for the oncogenicity of the T-DNA.

Please substitute the following paragraph on page 54, lines 18-28:

Once the binary vector of the cointegrative vector has been introduced into a suitable *Agrobacterium* strain (and cointegration has occurred), the next stage is to permit the *Agrobacterium* to infect plant cells. Various methods exist, including inoculation of intact plants with *Agrobacterium* cultures by injection, but the most widely used is to incubate discs cut from leaves of the target plant with an *Agrobacterium* culture. The bacterium will attack cells around the edge of the wounded leaf disc and transfer its T-DNA back into them. The leaf discs are then transferred to a suitable medium to select for transformation. The neomycin phosphotransferase gene is widely used, conferring resistance to aminoglycoside antibiotics, such as neomycin,

kanamycin, and G518. On a suitable selective medium, shoots form around the edges of the treated leaf discs. The shoots can then be regenerated into intact plants. See Howe, *Gene Cloning and Manipulation* (1995), Cambridge University Press, New York.